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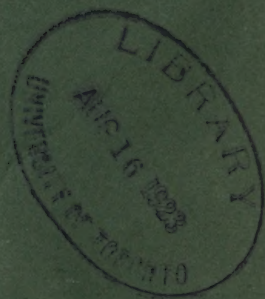
Imperial
Agricultural Research Institute, (Pusa)

Observations on the Morphology and Life-Cycle
of *Filaria recondita* Grassi

BY

M. ANANT NARAYAN RAO,

Ag. Assistant Professor, Madras Veterinary College



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THE GOVERNMENT OF INDIA, CALCUTTA

Observations on the Morphology and Life-Cycle of *Filaria recondita* Grassi.

(Received for publication on 6th October 1922.)

It is over half a century since microfilariæ were demonstrated in the general circulation of man, animals and birds, and this has given rise to a good deal of discussion as to the species of nematodes which are the mature forms of the microfilariæ. A few of the mature worms have been discovered and their life-history worked out, but the life-history of many others has not been published.

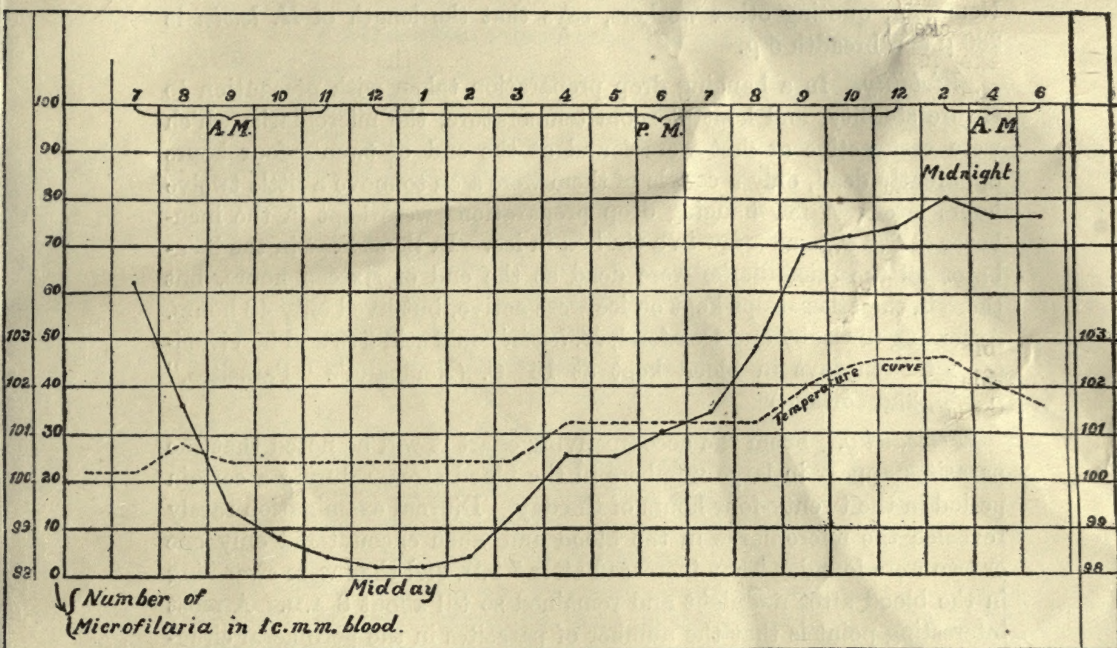
A dog harbouring microfilariæ in its general circulation came under the notice of the writer during the last week of December 1921, but unfortunately the dog died soon after and the chance of studying these parasites was lost. This was the first time that the writer found microfilariæ in the blood of a dog in the hospital of the Madras Veterinary College. In the month of January 1922, another case of "filariasis" was found and every opportunity was taken for making observations. The history of these two cases showed that the first dog was brought to Madras from Baluchistan and the second one from the Punjab.

Source of material. Observations were made on the few cases harbouring microfilariæ among the in-patients in the dog-ward of the Madras Veterinary College Hospital. From December 1921 till June 1922 seven cases were detected out of 299 dogs admitted as in-patients during that period. The microfilariæ from these seven different cases all appeared to be the same. The parasite was not found in even one dog out of 555 treated as out-patients during the same period. The writer has had neither the time nor facility to find out what percentage of street dogs in Madras harbour these parasites. Lewis says that he found as many as one in three in Calcutta but it is not known if this has been confirmed.

Description of the microfilariæ.

In a drop of fresh blood, the microfilariæ are snake-like organisms, long and fine, almost transparent, but when viewed with a high power lens a granular structure enclosed in a thin transparent cuticle is visible. This is best seen when the movements of the embryo are slow. They wriggle about, twist and untwist, and lash the blood cells near them

with their posterior ends. They often coil and uncoil like an earthworm attacked by ants. Another noticeable feature about the parasites is that they make long excursions in the drop of blood on the slide, but as often they catch hold of the cover glass by their anterior extremities and remain fixed for a long time while their bodies wriggle about in every direction. This characteristic feature agrees with the description of *Filaria recondita* or *Hæmatozoon lewisi*, given by Neumann on page 535 in his book on "Parasites" (2nd edition). They frequently shoot out a minute spine from the cephalic extremity and as quickly withdraw it once in every two or three seconds. This movement is analogous to the shooting out and retracting of the tongue of a snake. In a hanging drop of blood, in about four hours after making it, the movements of the microfilariæ slow down and at the end of twenty-four hours they are all more or less stretched out and apparently dead. Even at this stage they can be stained well with aniline dyes. These microfilariæ are not enclosed in a sheath. The anterior end is almost the same or a trifle smaller in diameter than that of the body. The cephalic extremity is rounded and does not contain any granules. The spine which often shoots out from the anterior extremity of the parasite is an interesting object. It is suggested that it probably plays an important part in bombarding and boring through the tissues. The ability of the worm to catch hold of a surface by its cephalic extremity further renders it easy to concentrate the bombardment at a particular point. The shooting out of this spine is only observed in fresh blood in hanging drops. It is never seen in the microfilariæ that have gained entrance to the thorax of a mosquito. In a stained specimen the granules in the body of the parasite are very well seen, and also three breaks in their continuity are brought out clearly. These granules are larger and more closely packed than those found in *Microfilaria Bancrofti*. They appear to be arranged spirally and not strewn haphazardly. The breaks in the granules, excepting the first one—the so-called nerve ring—are not visible from every point of view. In the fresh state, the transverse striation of the cuticle is not well seen, but in many of the stained specimens it is brought out very clearly (Figs. 1 and 2). Manson says that it is "probably produced by the longitudinal contraction of the body of the parasite throwing a very delicate and but slightly elastic cuticle into folds like the bellows of an accordian." My observations agree with this and it seems to explain why one finds so much difference in the measurements of the microfilariæ, those showing folds always measuring less than those without them. Thinking that fixation with alcohol caused contraction, I allowed the slides to dry in the air, then hæmolysed the smear with distilled water and measured the microfilariæ while it was still wet. There was



Temperature and periodicity curve of *Filaria recondita* Grassi.

still the same difference however. One can very well compare the contractile movements of these parasites with those of the trunk of an elephant when it shortens its trunk. The so-called genital cells are visible in the fresh specimen, but are not well marked in a stained one. Fülleborn's vital staining method was adopted for live embryos and the stain was seen to diffuse within the parasite through the "V" spot. This seems to confirm the idea that the "V" spot forms part of a rudimentary excretory or water vascular system. The posterior end gradually tapers to a fine point. The granules extend almost to the end of the tail.

Measurements. The length of the microfilariae in fixed and stained films ranges from 221 to 225 microns and the breadth from $4\frac{1}{2}$ to $5\ \mu$. In a dry and unfixed film the length ranges from 230 to 256 μ , but the breadth remains the same as in the stained films. The parasites can, with some difficulty, be measured when they are alive, but the measurements can only be approximate. The live specimens are found to vary from 230 to 270 μ in length and from $4\frac{1}{2}$ to $5\frac{1}{2}\ \mu$ in breadth.

Neumann, quoting other workers, says that the length of *H. lewisi* is 280 μ and breadth 5 μ .

Viability. In a hanging drop preparation taken with precaution to ensure sterility, and kept at room temperature, the microfilariae which were very active at first were found at the end of twenty-four hours apparently dead, only a couple of them were seen to move a little twelve hours later. A few hanging drop preparations were kept in the incubator at 37° C., and a few in a tin box in ice. In those kept in the incubator all the microfilariae were dead at the end of sixteen hours, but those in the other drops kept on ice were active, but lived only 48 hours. According to Gruby and Delafond, *H. lewisi* could still live and be vivacious after ten days in blood kept at 15° C. (Neumann's "Parasites," p. 536, 2nd edition.)

Periodicity. From the accompanying chart it will be noted that the parasites appear in large numbers in the blood stream during a certain period in the twenty-four hours of the day. Diurnal examination rarely revealed the microfilariae in the blood and when encountered only one or two were found. They increased after 7 P.M. and they were swarming in the blood after midnight and remained so till about 8 A.M. A most interesting point is that the number of parasites in the peripheral circulation is greater than is usual at mid-day if the dog is kept in a dark room during the whole day. Exposure to the sun or exercise reduces the number of microfilariae in the peripheral circulation.

Enumeration of microfilariae in the various organs of the body.

The largest number of microfilariae were seen in the blood squeezed out of the lungs, the liver stands second, the kidneys stand third and the glands and spleen come last. The arteries and veins of the brain contained almost the same number as found in the drop of blood from the kidneys. The urine of every case harbouring these parasites was centrifuged and examined, always with negative results. Manson says that in the human being the largest number of microfilariae were found in the sections of lungs, but in the dog the largest number were found in the sections of the liver. (Fig. 4.) In these sections they were found in the capillary spaces. Taking into consideration the habit of these microfilariae in clinging to a cover glass in a hanging drop of blood there seems to be every likelihood of the capillaries being blocked by them, especially if a few of them become entangled to form a plug. Rivolta is therefore right, perhaps, in stating that these parasites do block up capillaries, but Neumann says that it is improbable that the embryos exercise an embolic action because of their small size.

Post-mortem examination and morbid anatomy.

Three *post-mortem* examinations were made on dogs which had harboured this parasite and the changes observed were similar in each of them. All the internal organs were anæmic. There was a certain amount of dilatation of both sides of the heart. The liver was enlarged and showed on the surface whitish areas varying from the size of a mustard seed to the size of a walnut. On section similar areas were also found in the interior of the liver as well. The picture of the liver was exactly like the one illustrating "Infraction of the liver" given in the "Text-book of Pathology" by Delafield and Prudden. Microscopical examination of sections showed large areas of caseation surrounded by a zone of congestion. The caseated portions were also surrounded by a well-marked zone composed of fibro-blastic tissue. In the other portions of the liver the central veins were dilated showing chronic venous congestion. Pigment granules in the cells were also seen here and there.

The spleen was considerably enlarged, the kidneys were pale and larger than normal. The microscopical examination of a section of the kidney showed degeneration involving the parenchymatous tissue. The interstitial substance showed proliferation though not to a marked extent. Leucocytic infiltration was also seen in the interstitial tissue. The sub-lumbar lymphatic glands were much enlarged—each was almost the size of a small orange. On section the glands were pink in colour and showed a few hæmorrhages.

Microscopical examination of sections showed hyperplasia and in some places caseation. The large blood vessels of the body did not show much change excepting a slight increase of thickness in the fibrous coat.

Intermediary host. With due respect to the work of eminent scientists like Grassi, I venture to suggest that the development of *Hamatozoon lewisi* of the dog takes place in the mosquito *Culex fatigans*. Near the kennels, where one of these dogs harbouring *H. lewisi* was kept, a few mosquitos—*Culex* and *Stegomyia*—were caught and dissected. Over 30 per cent. of the *Culex* mosquitos contained microfilariae in their midgut, but very few of the *Stegomyia* showed them, and then only in very small numbers. The number in the *Culex* mosquitos ranged from one to fifty-five in each. No time was lost in breeding these two varieties of mosquitos and experimenting with them in an attempt to settle the question as to whether the microfilariae they contained were from man, other animals or birds. A large number of each of these home-bred mosquitos were made to bite a dog harbouring these parasites and then the mosquitos were kept alive by feeding them on plantain juice, which they seem to like very much and thrive on. A few of these mosquitos

were dissected daily for nearly three weeks. By the end of that time the *Stegomyia* did not show developmental forms and were discarded.

Three hours after biting the dog a couple of *Culex* mosquitos were dissected and their midgut was crushed in normal saline solution on a slide and observed under a microscope. The embryos they contained resembled those seen in the peripheral blood of the dog (Fig. 8) but they were sluggish in movement and seemed to have given up the habit of catching hold of the cover glass with their cephalic extremities. This is probably because they were surrounded by thickened blood. A few of these shot out spines and retracted them as quickly as they do in dog's blood. Eight hours after feeding, the microfilariae in the mosquitos' midgut looked stouter though not longer than the ones found in the circulation. (Fig. 7.) The cutaneous covering of the body looked thickened and when the embryo bent on itself the inner curve folded up with a dent analogous to the one found in a partly inflated inner tube of a bicycle when folded on itself, and when the parasite straightened the dent jerked out straight. After 36 to 48 hours all of them had disappeared from the midgut and had migrated into the thoracic muscles of the mosquitos. There they lay between and parallel to the muscle fibres. Seven to nine days after biting the dog, the thoracic muscles of the mosquito contained short thick "larval" forms with a short prolongation at one end. Occasionally this prolongation or "tail" of the larva moved to and fro rapidly. The movements of the larva itself were very slow. It bent on itself and straightened in moving forwards. The cells in its interior were arranged in two rows. I found only 9 per cent. of the mosquitos experimented with showing the developmental forms in their muscles. (Figs. 9, 10 and 11.)

Measurement (in micron) of microfilariae in the gut of mosquito 8 hours after feeding.

	I	II	III	IV	V
Head end to nerve ring	40	38	42	40	40
Nerve ring to "V" spot	32	34	34	32	30
"V" spot to tail spot	100	96	96	102	100
Tail spot to end of tail	80	84	82	80	84
Total length	252	252	254	254	254

The breadth varied from $5\frac{1}{2}$ μ to $6\frac{1}{2}$ μ .

On the 15th day in the mouth of one mosquito, out of the six dissected, two long larvæ, each with a rudimentary alimentary canal extremity, were found. Each resembled very closely the illustration shown

in the book on "Tropical Diseases," by Sir Patrick Manson in connection with human filariasis. They were about half a millimeter in length. These forms were unfortunately lost while trying to stain them. Sections of a large number of other mosquitos which had been fed on an infected dog were also cut and examined after staining them. The larvæ in sections or in saline solution were difficult objects for photography, because it was not very easy to focus the whole larva in thick sections, nor was it easy to focus an almost transparent object in saline solution with the improvised apparatus I had for the work. Many workers have pointed out that the developed larvæ migrate towards the head of the mosquito and after reaching it remain coiled up inside the root of the proboscis, under the cephalic glands or they may even find their way into the labium. Labredo's method of subjecting the head of a live mosquito to pressure in normal saline solution was followed, and it was observed that the larvæ being dealt with escaped from the labium and also from near the root of the proboscis. In the accompanying photograph of the head of a mosquito I saw two larvæ escaping from a breach in the labium, one of which escaped altogether and the other remained as seen in the photograph. This mosquito had been fed on an infected dog 17 days previously (Fig. 12).

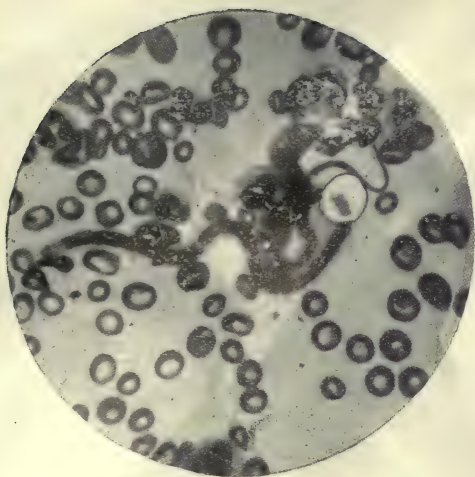
Concluding remarks.

The blood of many dogs brought to the College Hospital for treatment had been examined for various diseases prior to December 1921, but no cases of filariasis were detected until then. Except the first two cases, the others occurred in dogs that were brought to the hospital for other reasons and kept in contact with the first two cases for periods varying from a fortnight to a month. It seems feasible, therefore, to suppose that these five cases acquired the parasite after their admission into the hospital, and my observations lead me to believe that they did this through the agency of *Culex* mosquitos.

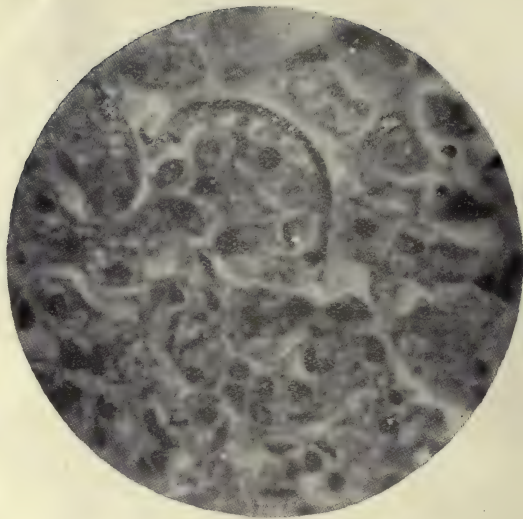
Grassi and Calendruccio say that they found the embryos of *H. lewisi* in the dog flea, cat flea, *Pulex irritans* and even in the tick *R. sanguineus*. This, I believe, has not been confirmed by other workers. I have dissected and sectioned many dog fleas collected from the infected dogs, but I did not find anything resembling the development forms of *H. lewisi* in them. It is possible, of course, for many blood-sucking parasites to ingest these microfilaria, but they may not develop in all of them.

I take this opportunity of thanking Mr. J. G. Cattell, M.R.C.V.S., D.V.S.M., who was then in charge of the College Laboratory, for so very kindly giving me valuable hints and encouragement.

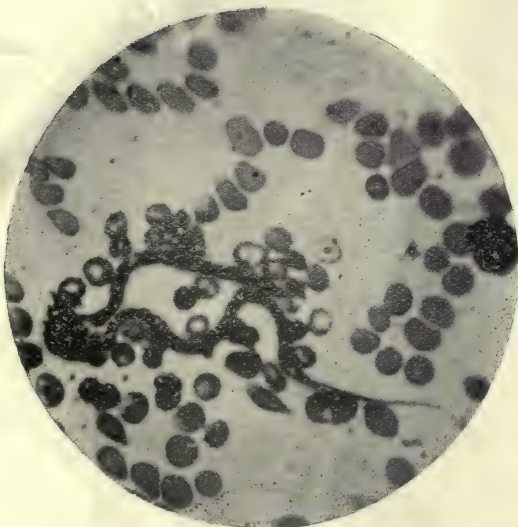




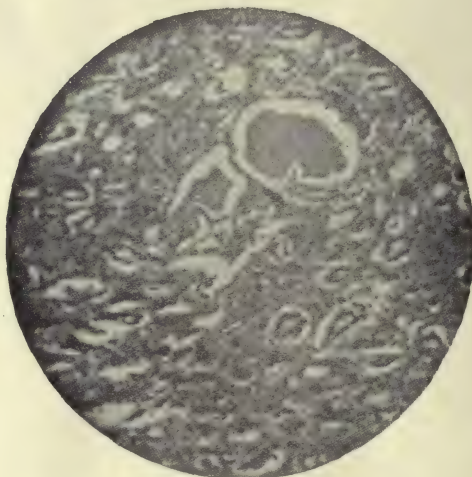
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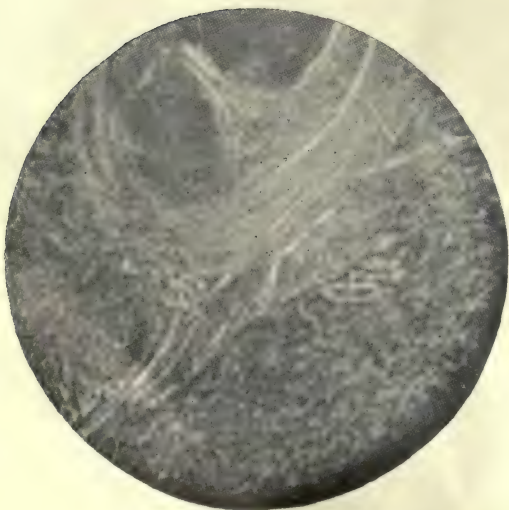
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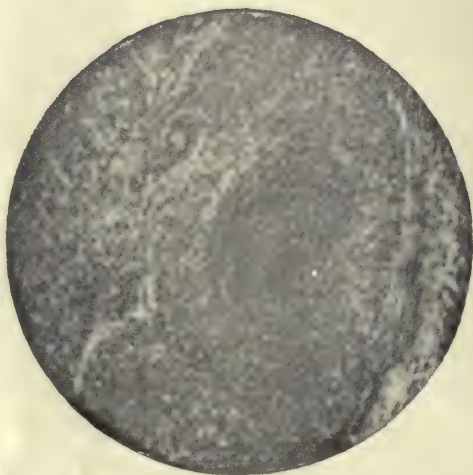
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Explanation of figures.

FIG. 1. *Filaria recondita* Grassi in the peripheral circulation. Note the spiral arrangement of the granules, clearly seen at the head. Note the striation at the tail. $\times 850$.

FIG. 2. *Filaria recondita*. Note the striation at the tail, nerve ring, and tail spot. $\times 850$.

FIG. 3. Section of the liver showing necrosis, caseation and degeneration of liver cells.

FIG. 4. Section of the liver showing a microfilaria in the capillary space. A portion of the parasite is seen. Note the granular changes in the protoplasm of the liver cells. $\times 650$.

FIG. 5. Section of the kidney showing degeneration of the glomeruli, catarrhal changes in the tubules and cell infiltration in the fibrous tissue.

FIG. 6. Section of the lymphatic gland showing caseation and proliferation of fibrous tissue.

FIG. 7. Microfilaria from the gut of a mosquito eight hours after feeding. $\times 850$.

FIG. 8. Microfilaria from the gut of a mosquito three hours after feeding. The parasite shows striation at the tail end though not well marked in the photograph. $\times 550$.

FIG. 9. Larva from a teased thoracic muscle of the mosquito in normal saline solution.

FIG. 10. Larvæ in the section of the thoracic muscle of a mosquito seven days after feeding. $\times 850$.

FIG. 11. Larva in the thoracic muscles of a mosquito twelve days after feeding. $\times 850$.

FIG. 12. Head and proboscis of a *Culex* mosquito showing larvæ of *F. recondita*; one has almost come out of the labium and another from near the base of the proboscis. The latter is damaged a little. The mosquito was fed on an infected dog seventeen days previously. $\times 350$.

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The third exercise is a study of the nature of the soul. It is a study of the soul as it is, not as it appears to be.

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The fifth exercise is a study of the nature of the mind. It is a study of the mind as it is, not as it appears to be.

The sixth exercise is a study of the nature of the will. It is a study of the will as it is, not as it appears to be.

The seventh exercise is a study of the nature of the emotions. It is a study of the emotions as they are, not as they appear to be.

The eighth exercise is a study of the nature of the intellect. It is a study of the intellect as it is, not as it appears to be.

The ninth exercise is a study of the nature of the senses. It is a study of the senses as they are, not as they appear to be.

The tenth exercise is a study of the nature of the passions. It is a study of the passions as they are, not as they appear to be.

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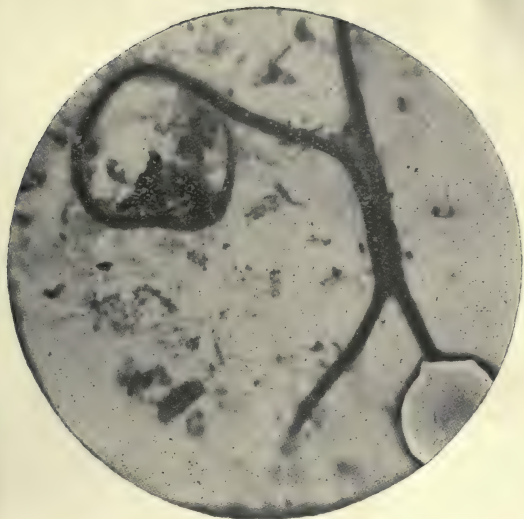
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The sixteenth exercise is a study of the nature of the sciences. It is a study of the sciences as they are, not as they appear to be.

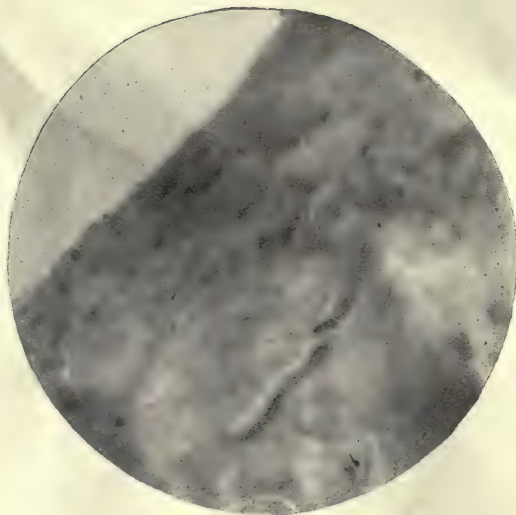
The seventeenth exercise is a study of the nature of the arts. It is a study of the arts as they are, not as they appear to be.

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The nineteenth exercise is a study of the nature of the occupations. It is a study of the occupations as they are, not as they appear to be.



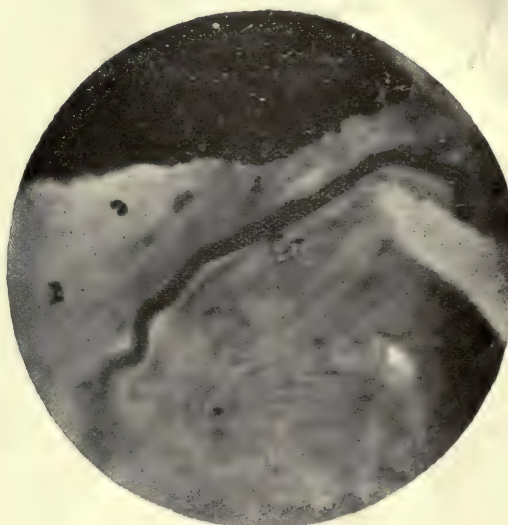
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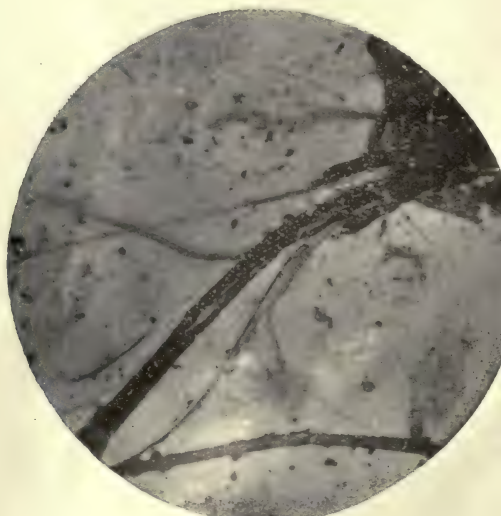
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